Biochimica et Biophysica Acta 1787 (2009) 681–690

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Mitochondrial ABC proteins in health and disease

Ariane Zutz [a], Simone Gompf [a], Hermann Schägger [b], Robert Tampé [a,*]

[a] Institute of Biochemistry, Biocenter, Goethe-University, Max-von-Laue-Str. 9, D-60348 Frankfurt a.M., Germany
[b] Gustav-Embden-Zentrum of Biological Chemistry, Medical School, Goethe-University, Theodor-Stern-Kai 7, D-60590 Frankfurt a.M., Germany

ARTICLE INFO

Article history:
Received 23 December 2008
Received in revised form 12 February 2009
Accepted 13 February 2009
Available online 24 February 2009

Keywords:
Heme biosynthesis
Intracellular transport
Iron homeostasis
Iron–sulfur cluster assembly
Mitochondrial targeting
Oxidative stress

ABSTRACT

ABC transporters represent one of the largest families of membrane proteins that are found in all three phyla of life. Mitochondria comprise up to four ABC systems, ABCB7/ATM1, ABCB10/MDL1, ABCB8 and ABCB6. These half-transporters, which assemble into homodimeric complexes, are involved in a number of key cellular processes, e.g. biogenesis of cytosolic iron–sulfur clusters, heme biosynthesis, iron homeostasis, multidrug resistance, and protection against oxidative stress. Here, we summarize recent advances and emerging themes in our understanding of how these ABC systems in the inner and outer mitochondrial membrane fulfill their functions in important (patho) physiological processes, including neurodegenerative and hematological disorders.

1. Introduction

ATP binding cassette (ABC) transporters belong to one of the most abundant families of integral membrane proteins found in all three kingdoms of life. Members of the ABC family play a major role in many cellular processes and mediate the active transport of a vast variety of solutes (e.g. amino acids, polysaccharides, peptides, lipids, drugs, antibiotics, toxins) across cellular membranes [1,2]. Although ABC proteins are represented in all organisms analyzed so far, their expression profiles and substrate specificities are highly diverse and differ in dependence of the specific cellular growth requirements and environmental influences. For instance, the genome of *Agrobacterium tumefaciens* encodes for 153 ABC proteins [3,4], while in *Saccharomyces cerevisiae* and human, 22 and 45 ABC transporters are present [5,6].

ABC transporters can be divided into two classes: i) ABC exporters that mediate e.g. the extrusion of toxic compounds (*self-defense*), and ii) ABC importers that primarily function as uptake systems for nutrients (*metabolism*). ABC importers typically recruit an extra substrate-binding protein that complexes the solute and hands it over to an outward-facing conformation of the ABC importer [7]. In eukaryotes, ABC transporters are located in the plasma membrane as well as in intracellular membranes of the endoplasmic reticulum (ER), the *Golgi* apparatus, lysosomes, peroxisomes and mitochondria. Almost all eukaryotic ABC transporters function as exporters by translocating a wide range of unrelated substances across cell membranes to an external compartment (lumen of the ER, peroxisomes, lysosomes, *Golgi*, or inner mitochondrial space). Their functions are linked to important cellular processes. In *S. cerevisiae* and other fungi, several ABC proteins mediate resistance against antimicrobial compounds and stress conditions [6]. Human ABC proteins are involved in a number of (patho) physiological processes, such as ion homeostasis of epithelial cells (cystic fibrosis transmembrane conductance regulator, CFTR), the regulation of insulin release from pancreatic beta cells (sulfonylurea receptor, SUR1/2), bile acid, lipid and cholesterol secretion, or antigen processing and presentation (TAP1/2). In addition, ABC transporters play a prime role in extruding chemotherapeutic drugs causing multidrug resistance of cancer cells. In this review, we will focus on mitochondrial ABC transporters, which regulate mitochondrial and cellular processes. According to the endosymbiotic theory, mitochondria developed from α-proteobacteria, in particular *Rickettsiales* that had become enclosed by Archaea early in evolution. Surprisingly, mitochondria harbor only a limited number of up to four ABC transporters compared to bacteria. In addition, mitochondrial ABC proteins have no homologues in bacteria; except the ones found in *Rickettsiae* and related bacteria. Furthermore, the role of ABC transporters in mitochondria appears to be very different from those in bacteria, leading to the suggestion that the mitochondrial ABCs evolved rather specific functions in the eukaryotic cell.

2. Mitochondrial ABC systems — a first inspection

Mitochondria are dynamic organelles that are involved in a number of essential cellular processes (Fig. 1). They play a focal role

Abbreviations: ABC, ATP-binding cassette; ATM, ABC transporter of mitochondria; NBD, nucleotide-binding domain; TMD, transmembrane domain; ER, endoplasmic reticulum; MDL, multidrug resistance like; MDR, multidrug resistance; IMM, inner mitochondrial membrane

* Corresponding author. Tel.: +49 69 798 29475, fax: +49 69 798 29495.
E-mail address: tampe@em.uni-frankfurt.de (R. Tampé).
URL: http://www.biochem.uni-frankfurt.de (R. Tampé).

0005-2728/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2009.02.009

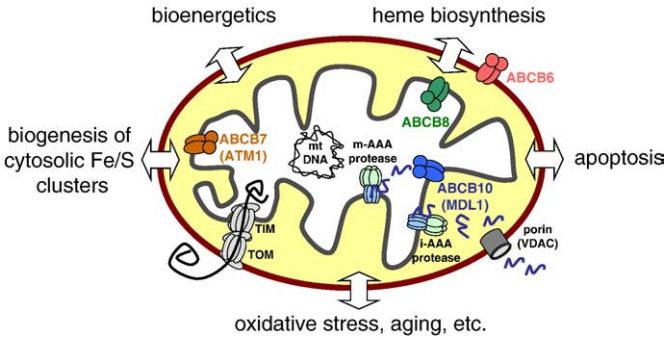

Fig. 1. Mitochondrial functions and mitochondrial ABC transporters.

in energy metabolism as the majority of the cellular ATP is generated via oxidative phosphorylation. Mitochondria are further involved in regulation of cellular iron homeostasis including the biogenesis of iron–sulfur clusters as well as heme biosynthesis. In addition, mitochondria are the major source of reactive oxygen species (ROS), which play a key role in ROS signaling and aging, but can also lead to severe cell damage. In higher eukaryotes, mitochondria play a major role in the regulation of apoptosis. Based on these vital processes, human diseases are linked to mitochondrial dysfunction. For instance, a reduced ATP level is associated with Parkinson's and Alzheimer's disease [8,9], while mutations in mitochondrial DNA can cause MELAS (Mitochondrial Encephalopathy and Lactic Acidosis with Stroke like episodes) or Leigh's syndrome [10,11]. Dysfunction of mitochondrial ABC proteins is directly linked to mitochondrial diseases. For example, the mitochondrial ABC transporter ABCB7 is involved in cellular iron homeostasis and causes a rare type of X-linked sideroblastic anemia with cerebella ataxia (XLSA/A) [12]. In addition, ABCB7 may play a role in RARS (Refractory Anemia with Ring Sideroblasts) [13], a myeloid malignancy that can transform to acute leukemia. Another mitochondrial ABC transporter ABCB8 appears to protect cells against ischemia and oxidative stress and is therefore predicted to have a cardio protective role in humans [14].

To date, four members of mitochondrial ABC systems have been identified, which most likely act as exporters. ABCB7/ATM1, ABCB10/MDL1 and ABCB8 are ABCB10/MDL1 and ABCB8 are localized in the inner mitochondrial membrane, while ABCB6 is found in the outer mitochondrial membrane. Mitochondrial ABC proteins belong to the

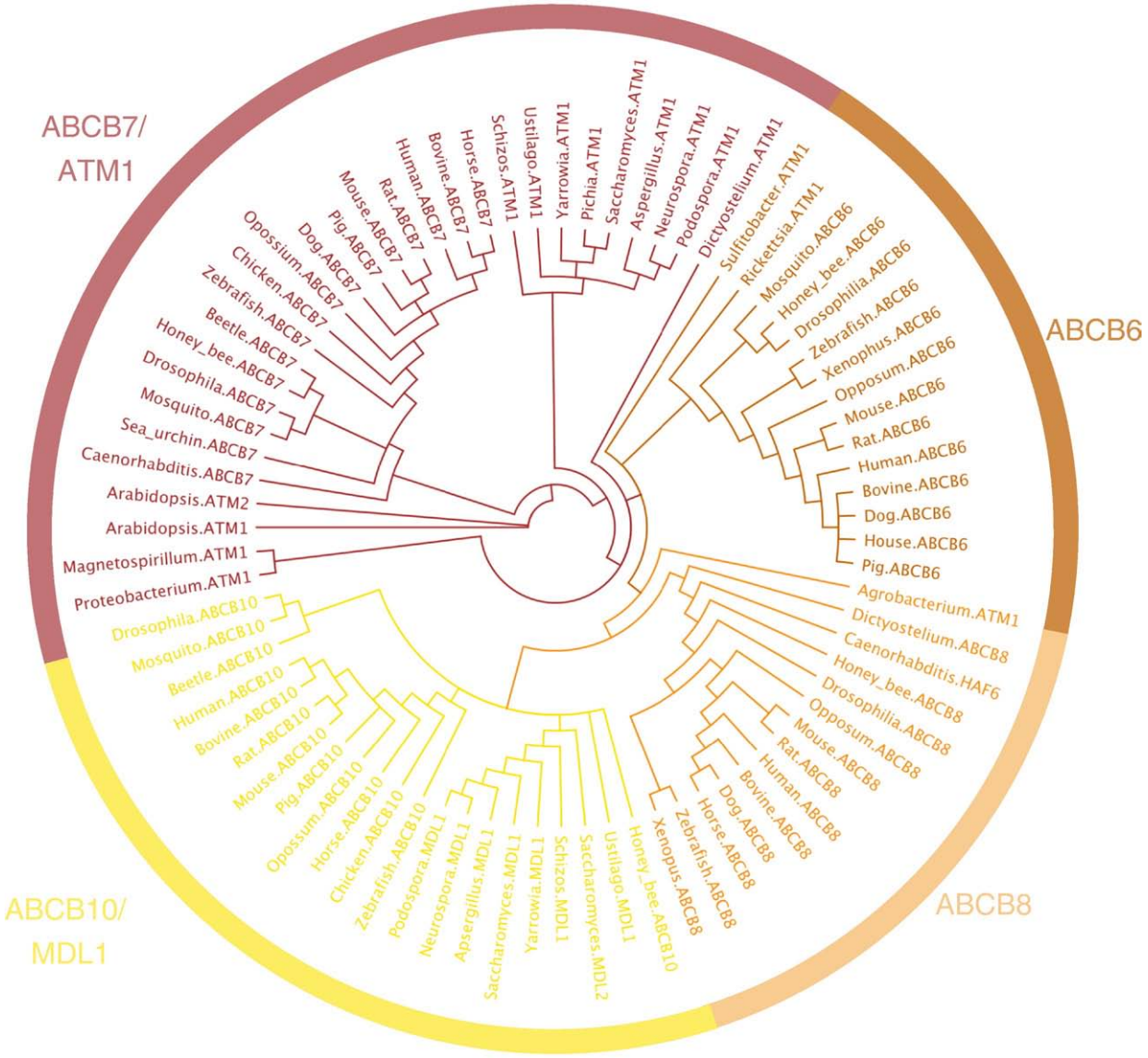

Fig. 2. Phylogenetic analysis of mitochondrial ABC transporters. Members of the subfamily ABCB7/ATM1, ABCB10/MDL1/2, ABCB8 and ABCB6 were aligned by ClustalW2 [87]. The phylogenetic tree was drawn by Geneious Pro 4.5. The sequence alignment including the accession number can be obtained from the authors.

subfamily B (MDR/TAP subfamily), including the multidrug transporter MDR1 (P-glycoprotein; ABCB1), the antigen transport complex TAP1/2 (ABCB2/3), and the yeast mating factor transporter Ste6. In baker's yeast, ATM1 (YMR301C), MDL1 (YLR188W) and MDL2 (YPL270W), which are localized to the inner mitochondrial membrane, can be divided into two classes based on their structural and functional properties: ATM1 is involved in regulation of cellular iron homeostasis and in cytosolic iron–sulfur cluster biogenesis [15]. The two closely related yeast proteins MDL1 and MDL2 represent the second class of mitochondrial ABC proteins. MDL1 has been proposed to be a peptide exporter similar to TAP1/2 [16], whereas the function of MDL2 is unknown. Homologues of these yeast mitochondrial ABC proteins are found in all eukaryotes (Fig. 2). In mammals, ABCB7 and ABCB6 (MTABC3) have been identified as close homologues of ATM1, implying a putative role of both proteins in regulation of iron homeostasis, while ABCB8 (M-ABC1) and ABCB10 (M-ABC2, ABC-me) are homologues of MDL1 and MDL2.

3. Structural organization of mitochondrial ABC transporters

Mitochondrial ABC proteins exhibit the archetypical domain organization of ABC transporters, which consist of two transmembrane domains (TMDs) that provide the passageway of solutes across the membrane and two nucleotide-binding domains (NBDs) that energize the directional transport by cycles of ATP binding and hydrolysis (Fig. 3). In general, the two TMDs and two NBDs are encoded by one, two, three or four genes. ABC exporters are organized as full and half-transporters, where one TMD is fused to one NBD. In the latter case, either two identical or two different half-transporters must assemble in order to form a functional homo or heterodimeric complex. In addition, full-length ABC transporters, such as the multidrug resistance protein (MDR1, ABCB1) comprising a single polypeptide chain, are frequently found in eukaryotes. In contrast, bacterial ABC import systems, such as the vitamin B_{12} transporter $BtuC_2D_2$ or the maltose permease $MalK_2FG$ from *Escherichia coli*, form a heterotetrameric complex [17,18]. Remarkably, all mitochondrial ABC transporters examined so far assemble as homodimers of half-transporters [19,20]. A cleavable N-terminal mitochondrial targeting sequence is fused to the TMDs, mediating the posttranslational import of the membrane protein. In contrast and notable exception, the mitochondrial CcmA/B complex found in *Arabidopsis thaliana* shows a domain organization similar to bacterial ABC importers [21].

ABC exporters usually feature a core domain of 2×6 membrane spanning α-helices that align the translocation pathway for solutes.

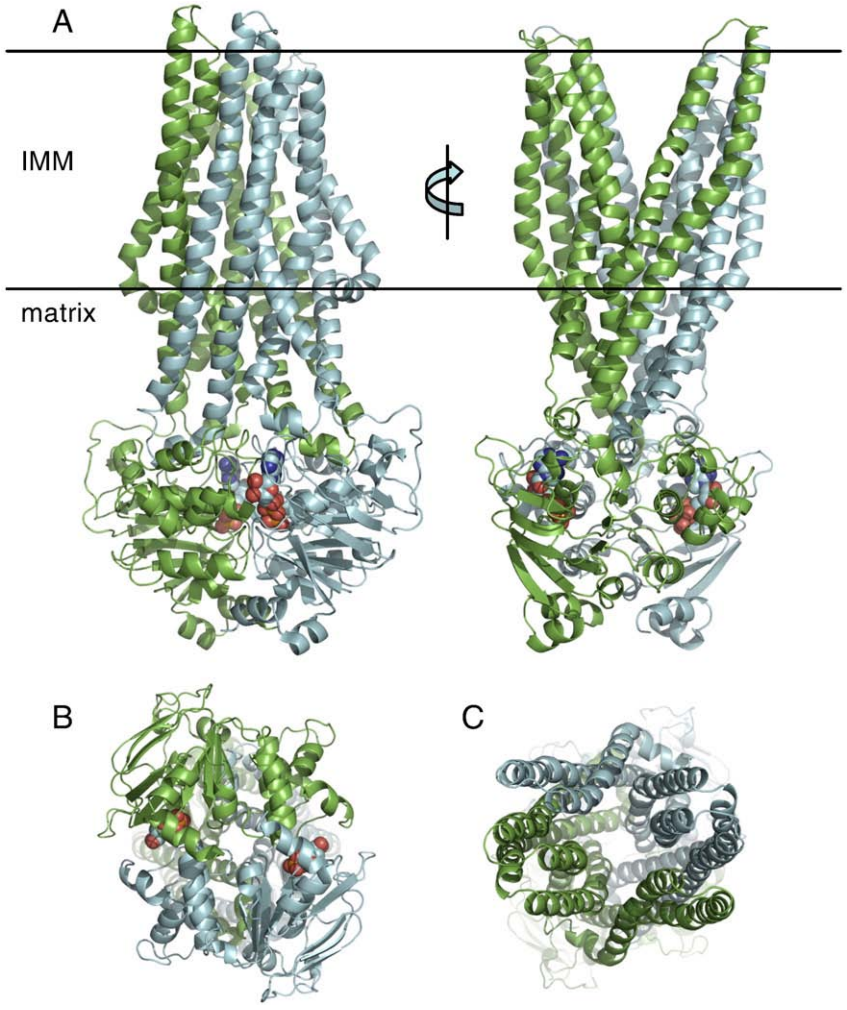

Fig. 3. Structural organization of mitochondrial ABC transporter. A 3D homology model of the homodimeric MDL1 complex was constructed based on the X-ray structure of Sav1866. Each half-transporter (cyan and green) was modeled on the corresponding subunit of the ADP-bound *S. aureus* Sav1866 homodimer (2HYD.pdb) [25]. The amino acid sequence of MDL1 and other homologous was aligned with that of Sav1866 using ClustalW2 (see Fig. 2). The alignment for mature (leader sequence-less) MDL1 [55] against Sav1866 revealed 31% sequence identity. Each MDL1 subunit was modeled separately by means of MODELLER v9.3 [88]. The half-transporters were dimerized to reproduce the Sav1866 subunit interface and refined to remove steric clashes [89]. In the 3D model (side view **A**), the NBDs form a sandwiched dimer by bound ATP (space filling model, view **B**) and the TMDs opened to the ER-luminal site, reflecting an outward-facing conformation (view **C**). The models are created by PyMOL.

Exceptions, such as the antigen transporter TAP1/2 (ABCB2/3) and TAPL (ABCB9), which cover an extra N-terminal TMD0 composed of four transmembrane helices acting as interaction hub, presumably developed late in evolution [22–24]. As revealed in the X-ray structure of the *Staphylococcus aureus* ABC exporter Sav1866 [25] and the 3D homology model of the yeast ABC transporter MDL1 (Fig. 3), the two TMDs are intertwined, where each 'wing' consists of helices TM1–TM2 from one subunit and TM3–TM6 from the other subunit. Notably, the transmembrane helices are connected by long, mostly α-helical cytosolic loops, which interact with the NBD of the opposite subunit via a short coupling helix. In the ATP-bound state, where both NBDs are in close contact to each other, the TMDs are arranged in an outward-facing conformation, in which the cytoplasmic site is closed while the extracellular part is open. This unique arrangement was also seen in the X-ray structure of the bacterial lipid ABC exporter MsbA, which was solved in three different conformations at lower resolution [26]. This structural analysis indicates large conformation changes, which may be required for solute translocation. Single particle EM analysis of the yeast mitochondrial transporter MDL1 revealed that the NBDs are separated, reflecting a V-shaped, inward-facing conformation [20]. Whether these large conformational changes are snapshots of different physiological states remains to be corroborated by future biochemical and biophysical approaches.

In contrast to the diverse structural organization of the TMDs of ABC systems, the NBDs are highly conserved, indicating that the mechanism of the NBDs in ABC importers and exporters are similar (Fig. 4). All NBDs of ABC systems contain the conserved Walker A (G–X–X–G–X–G–K–S/T) and Walker B motifs (Φ–Φ–Φ–Φ–D, where Φ represents a hydrophobic residue) [27], which mediate ATP binding [28–33] and coordination of the Mg^{2+} ion in the nucleotide-binding pocket [29,33,34]. A conserved glutamate, one residue downstream of the Walker B motif, acts as catalytic base [31]. This glutamate coordinates a water molecule that is proposed to attack the γ-phosphate of ATP [31,33,35]. The Q, D, and H-loop as well as the ABC signature motif (L–S–G–G–Q), also called the C-loop, is present in the NBD of ABC proteins [36]. The ABC signature motif, the hallmark of ABC proteins, connects the two NBDs via the bound nucleotide [35,37]. It is proposed that the Q-loop mediates the contact between NBDs and TMDs, while the D-loop is part of the contact interface between the two NBDs. The H-loop, also called switch region, consists of a conserved histidine that is proposed to be involved in the catalytic reaction [38].

X-ray studies of isolated NBDs, e.g. *E. coli* HisP [39], MalK [32], HlyB [38,40], *Sulfolobus solfataricus* GlcV [33], human TAP1 [30], and mouse CFTR [41] revealed that their structures are remarkably similar. Each NBD forms an L-shaped molecule that consists of two subdomains, the RecA-like (arm I) and a helical domain (arm II). The highly conserved Walker A and B motifs, as well as the H- and Q-loops are arranged in arm I, while the C-loop motif is localized in arm II (Fig. 4). Binding of ATP induces the dimerization of the two NBDs in a head-to-tail orientation, in which two ATPs are bound at the interface of the two monomers between the Walker A motif from one monomer (*cis*-site) and the C-loop (ABC signature) of the other monomer (*trans*-site) [31,35,37,42]. Comparing the different NBD structures, the largest conformational changes upon ATP binding and dimer formation occurs in the Q- and D-loop. ATP binding results in a rigid body movement of arm I towards arm II, while only minor differences are seen between the ADP bound and nucleotide free states.

4. Mechanism of ATP hydrolysis and transport

Despite detailed insight into the structure of ATP-binding cassettes, the mechanism of ATP hydrolysis and its coupling to vectorial transport across the membrane is still poorly understood. The mitochondrial ABC transporter MDL1 were instrumental to resolve the ATP hydrolysis cycle of isolated NBDs [42–44]. By using BeF_x trapping experiments as well as a MDL1 mutant with 1000-fold decreased ATPase activity as compared to wildtype, the formation and dissociation of the NBDs coordinated by nucleotides could be followed in single turnover experiments [42]. By tracing the molecular events

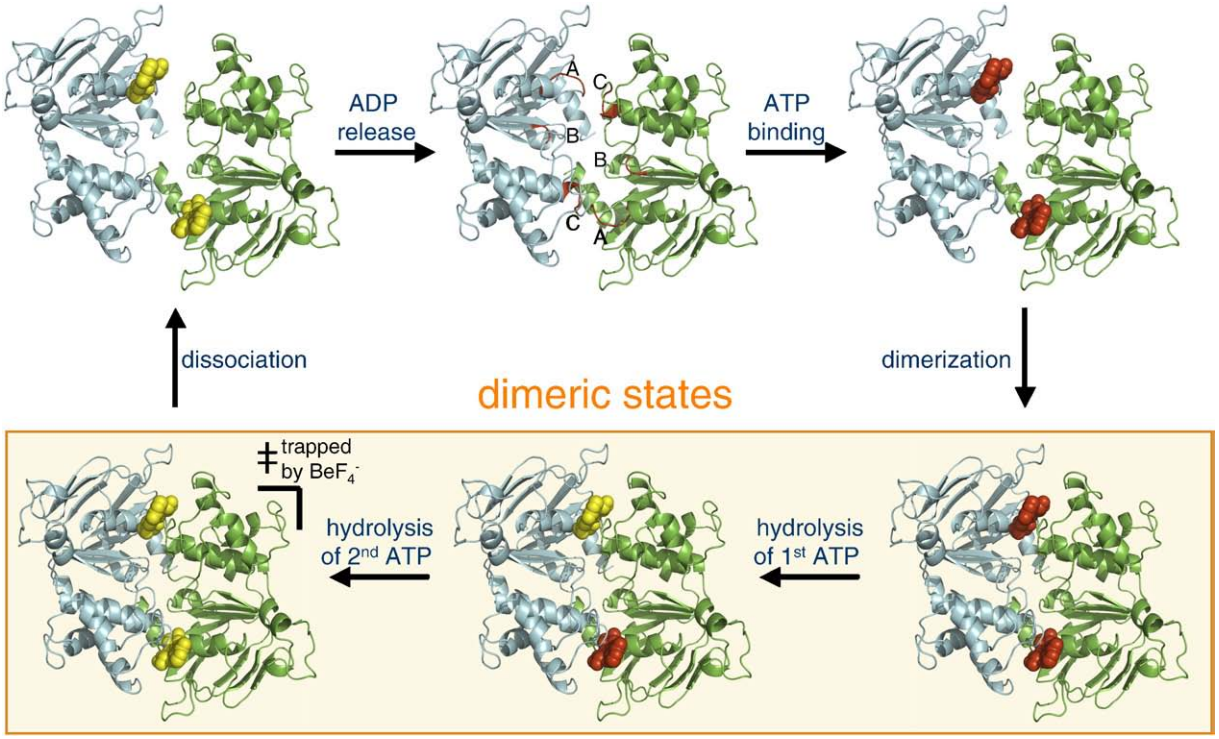

Fig. 4. The ATP hydrolysis cycle based on the processive clamp model. The hydrolysis cycle is derived from single turnover experiments of isolated NBDs (blue and cyan) of the homodimeric MDL1 complex [42–44]. ATP and ADP are depicted as space filled molecules, and colored red and yellow, respectively. The Walker A, B and C-loop motifs are indicated in red in the nucleotide free state. The models are schematic and created by PyMOL based on the homology model of MDL1 (see Fig. 3).

with [α-^{32}P] and [γ-^{32}P] ATP, three different intermediates of the NBD dimer could be resolved, which either contain two ATP molecules, one ATP and one ADP, or two ADP molecules trapped by BeF_x. Based on these data, a model of sequential hydrolysis (processive clamp) was proposed (Fig. 4). The hydrolysis cycle starts when ATP binds to each monomeric NBD. ATP binding is followed by dimerization of the two NBDs and sequential hydrolysis of both ATPs. After phosphate release, the dimer dissociates, leading to ADP release and exchange with ATP [42–44].

How the chemical energy of ATP is transferred to conformational changes of the TMDs is one of the key questions. Based on the X-ray structure of the full-length vitamin B_{12} ABC importer $BtuC_2D_2$, Locher et al. proposed that the region surrounding the Q-loop mediates a non-covalent interaction with the TMD [17]. This contact region, which appears to be architecturally conserved in ABC systems, is called the "transmission interface" and consists of coupling helices from the TMD that are embedded in a groove on the surface on the NBD. After ATP binding and dimerization of the NBDs, the distance between these coupling helices is decreased leading to a flipping from an inward to an outward-facing conformation (alternating access model). In ABC exporters, such as the mitochondrial ABC systems, the solute should be released to the opposite site of the cytosol or matrix (see Fig. 3), while bacterial import systems take up the solute from their docked binding proteins. At the end of the hydrolysis cycle, the TMDs flip back to the inward-facing conformation, followed by either recruitment of substrate in export systems or substrate release in import systems [45,46].

5. Posttranslational targeting of mitochondrial ABC proteins

Mitochondria contain about 800 to 1500 different proteins [47,48] whereas the vast majority of these (>98%) are nuclear encoded. They are synthesized as precursor proteins by free ribosomes and afterwards transported via the translocase of the outer membrane (TOM) into the organelle. To date, four classes of mitochondrial precursor proteins that enter the mitochondrial compartments by different import routes are identified. Integral membrane proteins can be translocated into the inner membrane via two transport mechanisms, the presequence pathway and the carrier pathway. For entering the presequence pathway inner membrane proteins possess a cleavable N-terminal mitochondrial targeting (leader) sequence, characterized by a positively charged amphipathic α-helix that is followed by a hydrophobic sorting signal. In contrast some integral membrane proteins, like the ADP/ATP carrier, comprise largely uncharacterized internal targeting signals, leading to integration into the inner mitochondrial membrane via the carrier pathway (for review see [49–52]).

ABC transporters possess highly hydrophobic TMDs and are thus predicted to enter the secretory pathway by co-translational insertion into the ER membrane. However, recent studies showed that the mitochondrial ABC transporters ABCB10 (ABC-me), ABCB7, and MDL1 are translated and partially folded in the cytosol, posttranslationally targeted to mitochondria and subsequently integrated into the inner mitochondrial membrane [53–55]. Remarkably, these ABC transporters display an unusual long N-terminal mitochondrial targeting sequence of 40–120 residues, which is dominated by positively charged amino acids [53–55]. A 59 amino acid long mitochondrial leader sequence was identified for MDL1, which is cleaved in the matrix during the posttranslational import [55]. Similarly, the mouse homologue ABCB10 comprises an exceptionally long leader sequence of 105 residues [54]. Here, the N-terminal part is critical for correct import, while the central region is essential for mitochondrial targeting. Notably, deletion of the leader sequence reroutes the ABC transporter MDL1 and ABCB10 to the ER membrane [54,55]. Importantly, these ABC proteins still form homodimeric complexes with functional and structural properties similar to the mitochondrial ABC transporters [54,55]. Interestingly, fusion of the leader sequence of mouse ABCB10 to the multidrug transporter MDR1 mediates a switch from co-translational ER targeting to post-translational mitochondrial import [56]. In contrast, the leader sequence of a soluble matrix protein as well as the deleted version of the ABC-me presequence could not guide ABC proteins to the inner mitochondrial membrane, showing that the unusual long leader sequence of these proteins is necessary for correct import and integration into the inner membrane [56]. However, the mechanistic details of the targeting sequence of mitochondrial ABC transporters are presently not known. It is proposed that the amount of positive charged amino acids, the unusual length, and/or a specific higher structure of the N-terminal domain are essential to avoid entering the classical secretory pathway, leading to efficient posttranslational import of hydrophobic membrane proteins into the inner mitochondrial membrane [54–57].

Most remarkably, ABCB6 is the only known ABC protein that resides in the outer mitochondrial membrane [58]. However, ABCB6 was also found in the classical secretion pathway, including the ER membrane, the *Golgi* apparatus and plasma membrane [59,60]. So far, it is unclear how ABCB6 is specifically targeted to different subcellular compartments. It has been proposed that specific structural requirements and not a specific amino acid sequence are necessary for integration of ABCB6 into mitochondria [59], as it was shown for other proteins that traverse the outer mitochondrial membrane [61].

6. ABCB7/ATM1 is essential for cytosolic iron–sulfur cluster biogenesis and iron homeostasis

ATM1 (ABC transporter of mitochondria) was the first mitochondrial ABC transporter to be identified [62]. The 70-kDa protein (690 aa) forms a homodimeric complex in the inner mitochondrial membrane [19,62]. Yeast cells lacking *ATM1* show a severe growth phenotype. They are only able to grow on fermentable carbon sources, suggesting a loss of oxidative respiration [62,63]. In addition, depletion of ATM1 results in 30-fold higher accumulation of mitochondrial iron [63]. Due to the redox properties of the non-ligated iron, reactive oxygen species are formed. In consequence of oxidative stress, a series of secondary effects occur in ATM1 depleted cells, causing the loss of the mitochondrial DNA (rho^0 phenotype), an increased content of glutathione, and deficiency of apo forms of heme proteins [62,63]. In contrast to the iron accumulation in the matrix, ATM1 depleted cells show evidence for starvation of cytosolic iron [64]. By tracing the incorporation of ^{55}Fe into the cytosolic iron–sulfur protein LEU1, Lill et al. could demonstrate that ATM1 plays a key role in maturation of cytosolic Fe/S cluster proteins [15], some of which are essential for yeast [65]. Taken together these results imply that ATM1 is involved in regulation of cellular iron homeostasis.

The global transcriptional responses to ATM1 depletion in *S. cerevisiae* were analyzed by DNA microarrays. Defects in ATM1 or other compounds of the iron–sulfur cluster assembly system strongly induce transcriptional response of more than 200 genes. ATM1 depletion activates the iron-responsive transcription factors AFT1/2, which regulate the high-affinity uptake of iron into the cytosol. As a second consequence, respiration and heme metabolism are repressed in cells lacking ATM1. Furthermore, genes involved in glucose acquisition are up-regulated to compensate the respiratory deficiencies of ATM1 depleted cells. Taken together, these data underline a role of ATM1 in the biosynthesis of heme and iron–sulfur clusters, and cellular iron homeostasis in general [66].

Despite its essential function in a number of physiological processes, the physiological substrate of ATM1 has unfortunately not been identified. ATM1 may translocate compounds, which directly or indirectly act as precursors or factors for the maturation of cytosolic iron–sulfur cluster proteins. Based on the observation that iron accumulates in ATM1 deficient mitochondria, it was first assumed that the transporter is involved in exporting an iron-chelating

molecule. However, ATM1 dependent iron transport has not been observed so far. Thus it is under discussion whether the accumulation of iron indeed is a prime consequence of ATM1 depletion. Recently, it was shown that compounds displaying free sulfhydryl groups, e.g. cysteine containing peptides such as glutathione, cofactors such as coenzyme A, or organic substances such as dithiothreitol, stimulated the ATPase activity of ATM1. Based on these findings it was speculated that ATM1 is involved in transport of cysteine enriched peptidic molecules that are putative substrates of the Fe/S cluster assembly machinery [67]. However, the physiological nature of ATM1 substrate remains cloudy and thus must to be a prime target for future investigations.

In mammals, two mitochondrial ABC transporters ABCB7 and ABCB6 have been identified to be structural and functional homologues of yeast ATM1 (Fig. 2). Human ABCB7 exhibits 49% sequence identity to ATM1 of baker's yeast. Interestingly, ABCB7 as well as ABCB6 appear to complement the growth defects of ATM1 depleted cells, implying a common role of these two mitochondrial ABC exporters in cellular iron homeostasis [53,68]. ABCB7 was mapped to the X-chromosome in mouse and human [69]. ABCB7 shows a ubiquitous expression pattern, with highest expression in muscle. Knockout studies in mice revealed that expression of ABCB7 is essential for early gestation in all tissues, with the exception of liver and endothelial cells [70]. Expression of mammalian ABCB7 in yeast restores the growth phenotype of ATM1 depleted cells [53]. The amount of mitochondrial cytochromes, the content of mitochondrial iron and glutathione in Δ*atm1*/ABCB7 yeast cells was found to be similar to that of the wildtype strain. Most importantly, ABCB7 can complement the function of ATM1 by mediating cytosolic iron–sulfur biogenesis. Further studies revealed that defects in ABCB7 inhibit the maturation of cytosolic Fe/S clusters in human cells, giving a possible link to a role of ABCB7 in XLSA/A, a human disease that is characterized by loss of cytosolic Fe/S cluster proteins, defects in heme metabolism and elevated contents of mitochondrial iron [12]. Apart from its essential function in the biogenesis of cytosolic Fe/S cluster proteins, a putative role of ABCB7 in hematopoesis was observed [71]. Recent studies revealed an interaction of ABCB7 with ferrochelatase, which catalyzes the last step of heme synthesis in the mitochondrial matrix by incorporation of Fe^{2+} into protoporphyrin IX. This suggests a putative role for ABCB7 in heme metabolism in erythroid cells [72]. Down-regulation of ABCB7 by siRNA in HeLa cells caused a strong reduction of cell proliferation combined with cytosolic iron deprivation. In ABCB7 depleted cells, an increase of mitochondrial iron that is not accessible by mitochondrial ferritin, an increased sensitivity to oxidizing agents and an accumulation of protoporphyrin IX was observed [73]. Collectively, these results imply a putative function of ABCB7 in regulation of iron incorporation into Fe/S clusters and heme, and a prime role in cellular iron homeostasis.

7. The role of mammalian ABCB6 in heme biosynthesis

ABCB6 (MTABC3), the second ABC transporter that exhibits strong sequence homology to ATM1 (Fig. 2), is ubiquitously expressed in rat and human tissues [68]. Surprisingly, ABCB6 was found in the outer mitochondrial membrane with its NBDs facing the cytosol, suggesting a possible function of ABCB6 in energy dependent mitochondrial import processes [58,59]. Although ABCB6 appears to be a mitochondrial outer membrane protein, it can complement the phenotype of ATM1 depleted yeast cells [68] and is therefore proposed to be an additional regulator of iron homeostasis in human cells. However, expression of ABCB6 in Δ*atm1* cells can only partially restore the mitochondrial iron level compared to wildtype level. As ABCB6 and ABCB7 are resident in different mitochondrial membranes, it appears unlikely that both ABC transporters represent functional homologues.

Apart from its putative role in iron metabolism, additional functions have been proposed for ABCB6. It was recently reported that mouse erythroleukemia (MEL) cells exhibit increased transcriptional and translation levels of ABCB6 due to elevation of cellular porphyrins [58]. ABCB6 binds heme and other tetrapyrrole carboxylates, indicating that the ABC protein may be involved in mitochondrial porphyrin uptake [58]. In addition, a putative role of ABCB6 in resistance to cytotoxic agents was reported. Analysis of the ABCB6 expression profile after drug treatment revealed that an increase of ABCB6 expression correlates with increased resistance to various drugs in different cancer cell lines [74]. Therefore ABCB6 is a candidate for mediating multidrug resistance (MDR) of cancer cells.

In addition to its mitochondrial localization, ABCB6 was also detected in the plasma membrane with an up-shifted molecular mass higher than the mitochondrial protein [59]. The reason for dual localization of ABCB6 is poorly understood but in agreement with reports that detected the ABC transporter also in red blood cells [75]. Recent studies revealed that ABCB6 is also found in the classical secretory pathway, in particular in the *Golgi* apparatus [60]. Notably, ABCB6 located either in the plasma membrane or mitochondria transports hemin and pheophorbide (PhA), implying a similar function of both proteins in transport of heme-like compounds. ABCB6 in the plasma membrane may protect cells from toxic effects of PhA [59], which is found in food [76]. The *Golgi* ABCB6 is proposed to transport porphyrin-related compounds, such as Foscan, a drug used in therapy of certain types of cancer [60], providing a possible link to ABCB6 induced multidrug resistance. The function of plasma membrane as well as *Golgi* localized ABCB6 has to be elucidated by further investigations.

8. Mitochondrial export of an ambiguous compound by MDL1/ABCB10

MDL1 was discovered in 1994 by a genetic screen in yeast [77]. MDL1 (695 aa) forms a homodimeric ABC exporter in the inner mitochondrial membrane [16,20,55]. MDL1 deletion did not display a phenotype [77]. Langer et al. proposed that MDL1 exports peptides that are derived from the mitochondrial quality control system into the inter-membrane space [16]. These degradation products should be released into the cytosol via the porin VDAC1, where they may be involved in the communication between mitochondria and their cellular environment. It has been shown that the release of peptides in the range of 0.6 to 2.1 kDa was reduced in Δ*mdl1* mitochondria compared to wildtype, and that over-expression of MDL1 in Δ*mdl1* cells restored this defect [16]. However, the physiological relevance of mitochondrial peptide release via MDL1 is under debate because mitochondria have evolved additional proteolytic systems that mediate extrusion of (poly) peptides out of mitochondrial matrix. Due to this fact it appears likely that MDL1 transports specfic peptidic factors, which are linked to mitochondrial processes.

To get more insight into the specificity of MDL1, substrate uptake and substrate-stimulated ATPase assays were performed with MDL1 reconstituted into proteoliposomes as well as with microsomes that contain leaderless MDL1 [20,55]. Collections of peptides that display a putative substrate for MDL1 were screened, including combinatorial peptide libraries [24,78,79], a set of defined peptides such as *N*-formylated peptides or fragments of mitochondrially encoded gene products as well as cysteine containing peptides that have been shown to stimulate the ATPase activity of ATM1 [67]. However, neither a substrate-stimulated ATPase nor specific transport activity could be observed, suggesting that MDL1 is indeed highly specific for a very small subset of compounds or even for modified peptides that are largely under-represented in the peptide libraries. We propose that MDL1 translocates peptidic substrates with additional factors, such as metal ions or sulfur compounds.

Next to the putative function in mitochondrial peptide release, it was proposed that MDL1 interacts with the F_O/F_1-ATP synthase [80], suggesting a putative role of MDL1 in regulation of respiratory

function in mitochondria. However, the interaction could not be confirmed in a detergent screen, including mild detergents such as digitonin [20]. This leads to the conclusion that the interaction of MDL1 and the F_O/F_1-ATP synthase in Triton X-100 micelles is unspecific. Of note, the co-purification of F-type ATPases has also been observed in case of other ABC transporters. Therefore, it is unclear whether MDL1 is directly involved in regulation of respiratory function by interacting with the F_O/F_1-ATP synthase.

By using a yeast complementation screen, it was demonstrated that high-copy expression of MDL1 partially restores the drastic phenotype of Δ*atm1* cells [64]. Notably, MDL1 targeted to the ER or MDL1 mutants, which are inactive in ATP binding or hydrolysis, do not rescue the growth phenotype of ATM1 depleted cells [55]. This implies that ATM1, ABCB7 and MDL1 have overlapping functions. In addition, expression of MDL1 in a Δ*atm1* background drastically increases resistance to H_2O_2 [64]. It was therefore proposed that the high copy suppression of *MDL1* is largely due to an effect on resistance to oxidative stress that occurs as a secondary consequence of iron accumulation, and not due to a role of MDL1 in delivering precursor molecules for cytosolic iron–sulfur cluster assembly.

To characterize the Δ*atm1*/MDL1s mitochondria, we analyzed their mitochondrial DNA by Southern blot analysis. Δ*atm1*/MDL1 cells still display the rho^0 phenotype of Δ*atm1* cells (Fig. 5A). In contrast to the nuclear or plasmid encoded *MDL1*, the mitochondrially encoded *COXI* and *COXIII* genes were not detectable in these cells, suggesting that the mitochondrial DNA is damaged or lost due to oxidative stress, which is caused by the elevated iron content in Δ*atm1*/MDL1 mitochondria. These findings were confirmed on the protein level by immunoblotting (Fig. 5B). Because of the lack of mitochondrially encoded proteins, the assembly of respiratory chain complexes is abolished as demonstrated by Blue-Native (BN) PAGE (Fig. 5C), suggesting that over-expression of *MDL1* does not restore the respiratory deficiency of Δ*atm1* cells. This is in agreement with the observation that Δ*atm1*/MDL1 cells are only able to grow on fermentable carbon sources [64]. Taken together, Δ*atm1*/MDL1 mitochondria are damaged by elevated levels of mitochondrial iron and oxidative stress. Thus, rather than protecting cells against oxidative stress, MDL1 and ATM1 have an overlapping substrate specificity with regard to the delivery of an essential compound for iron–sulfur cluster synthesis in the cytosol. Whether this compound

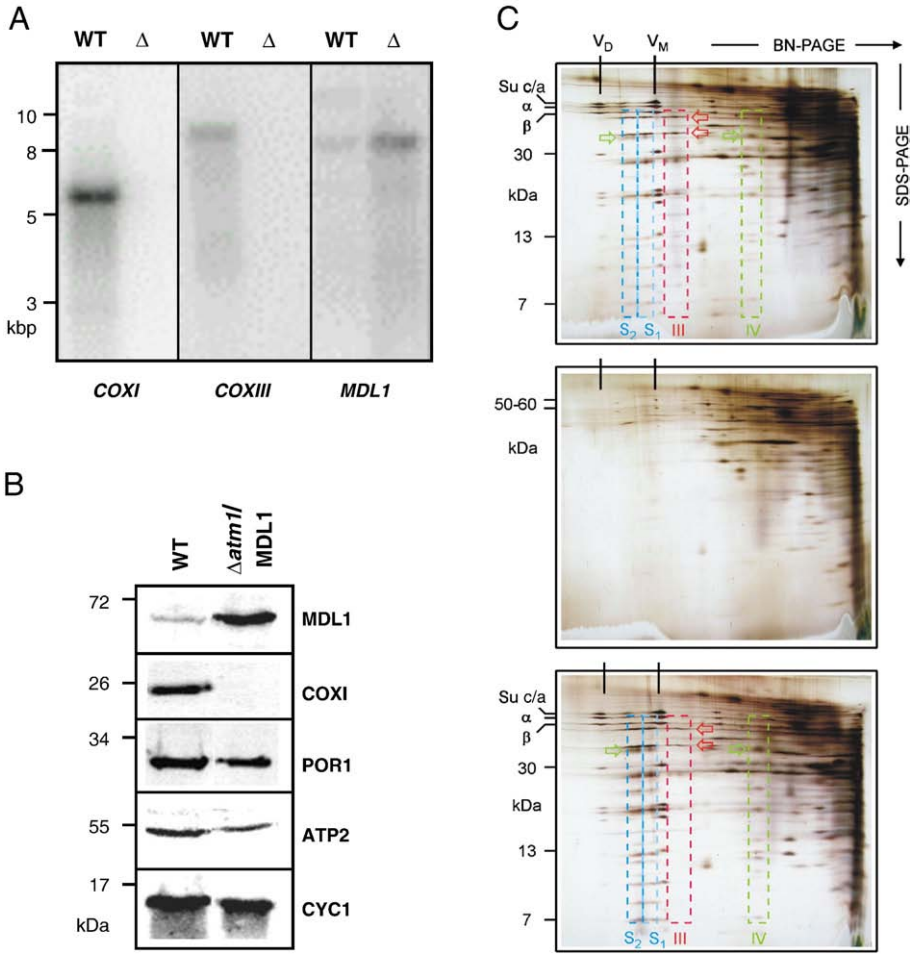

Fig. 5. Characterization of Δ*atm1*/MDL1 mitochondria. The following experiments were performed in the W303 genetic background. Δ*atm1*/MDL1+ATM1 yeast cells were grown on 5′-fluoroorotic acid selection plates to generate Δ*atm1*/MDL1 cells [55,64]. **(A)** Mitochondrial DNA of wildtype (WT) and Δ*atm1*/MDL1 yeast (Δ) was analyzed by Southern hybridization. 1 μg of genomic DNA digested by EcoRI was loaded in each lane. DNA probes labeled with [α-^{32}P]-dATP were used to detect mitochondrially encoded COXI and COXIII as well as the nuclear and plasmid encoded MDL1. **(B)** Total mitochondrial extracts (10 μg of protein) prepared from W303 and *Δatm1*/MDL1 yeast are analyzed by SDS-PAGE and immunoblotting using antibodies against POR1, ATP2, CYC1, COXI and MDL1. **(C)** Respiratory chain complexes of mitochondria isolated from WT (upper panel), *Δatm1*/MDL1 (middle panel), and Δ*atm1*/MDL1+ATM1 yeast (lower panel) were analyzed by Blue-Native-PAGE as described [90]. Mitochondria (100 μg of protein) were solubilized in digitonin at a detergent-to-protein ratio of 6 (w/w). Proteins were first separated on a linear acrylamide gel (4–13%) followed by Tricine SDS-PAGE (13%). In *S. cerevisiae*, complexes III and IV can be observed as super complexes (S_1: III_2IV_1, S_2: III_2IV_2). The monomer and dimer of the ATP synthase (complex V) are labeled with V_D and V_M. The green arrow shows COXI (subunit of cytochrome *c* oxidase) and the red arrow shows core proteins I and II of complex III. A ring of ten *c*-subunits and monomeric subunit *a* associate to a subunit *c*/*a* complex (Su *c*/*a*) as described in [91]. Notably, *Δatm1*/MDL1 mitochondria lack all respiratory chain complexes, compared to mitochondria of WT and Δ*atm1*/MDL1+ATM1 cells.

is a specific peptide, which functions as a precursor or factor for iron–sulfur cluster maturation, has to be elucidated in further investigations.

Secondary effects, caused by the elevated iron content, avert the analysis of MDL1 function in Δ*atm1*/MDL1 cells. Nevertheless, the genetic complementation screen appears very useful for the investigation of inactive ATM1 and MDL1 mutants [64] as well as for identification ATM1 homologues in other eukaryotes, as it was shown for ABCB7 and ABCB6 [53,68]. By using the complementation screen, we could identify human ABCB10, as a suppressor of ATM1, whereas ABCB8 cannot complement ATM1 function (S.G., A.Z. & R.T., unpublished data).

Human ABCB10 is expressed in most tissues, with highest expression level in the bone marrow [81], suggesting a putative role in hematopoesis [82]. Furthermore, mouse ABCB10 (ABC-me, ABC-mitochondrial erythroid) is mostly expressed in tissues that are involved in hematopoesis, such as bone marrow or fetal liver. ABCB10 gene expression is up-regulated during erythropoiesis, leading to the conclusion that this mitochondrial ABC protein may mediate transport functions that are linked to heme biosynthesis [83]. Whether the function of the homologous transporter MDL1 is also related to regulation of heme metabolism in *S. cerevisiae* and how this process is linked to the transport of a specific peptidic compound requires future investigations.

9. Elusive functions of MDL2 and ABCB8

Hardly anything is known about the physiological function of yeast ABC transporter MDL2. This closest MDL1 homologue (773 aa) is localized in the inner mitochondrial membrane and forms a homodimeric complex with a molecular mass of 300 kDa. MDL2 deficient yeast cells show a decreased growth on glycerol containing media compared to wildtype cells [16]. Recent studies revealed growth on oleate acid is also reduced in *MDL2* deleted cells [84], suggesting a possible role of MDL2 in regulation of mitochondrial lipid homeostasis.

Transcription profiles revealed that ABCB8 (M-ABC1) is expressed in most human tissues. The 60-kDa protein forms a higher molecular complex of 150 kDa under non-reducing conditions [85]. Co-immunoprecipitation as well as yeast 2-hybrid screens revealed that ABCB8 forms a complex with other mitochondrial proteins, including succinate dehydrogenase, inorganic phosphate carrier, adenine nucleotide translocator, and ATP synthase [86]. The macromolecular complex was identified as a mitochondrial ATP-sensitive K^+-channel that seems to be involved in protection of cells against oxidative stress [14]. However, the mechanism and architectural organization of the K^+-channel in mitochondria is unknown and therefore the function of ABCB8 is still unclear.

10. Conclusions and future perspectives

Despite their key role in the cellular iron homeostasis, including the biogenesis of cytosolic iron–sulfur and heme proteins, the substrate of all mitochondrial ABC systems has not been identified and remains a large mystery. In order to understand the physiology and pathogenesis of the processes, this unresolved issue should be of prime focus of future investigations. ATM1 from *S. cerevisiae* exhibits the most severe phenotype observed for ABC transporters in yeast. Homologues of ATM1 are found in all eukaryotes, underlining their essential function in iron metabolism. Remarkably, other mitochondrial ABC proteins, such as ABCB10, MDL1 as well as ABCB6 can rescue the drastic growth phenotype of ATM1 deleted yeast cells, implying that these mitochondrial ABC proteins act as a backup system. Furthermore, a putative role in heme metabolism has been proposed for ABCB7, ABCB10 and ABCB6. Due to their conserved homodimeric architecture, mitochondrial ABC transporters represent interesting targets for structural studies in order to obtain detailed insight into the molecular mechanism of the energy coupling via ill-defined conformational changes. The fascinating promiscuity in subcellular targeting, in particular of ABCB6, requires further investigation and may disclose novel strategies of processing and trafficking of these ABC transporters also in regard to its functional regulation under various cellular conditions. In this context, the function of unusually long mitochondrial leader sequence should be addressed. Future work may imply novel proteomics approaches to identify interaction partners and regulation networks to gain a mechanistic understanding of complex diseases including metabolic, neurodegenerative and hematological disorders.

Acknowledgements

We would like to thank Drs. David Parcej, Chris von Does and other members of the lab for helpful discussions and Drs. Peter Rehling (University Göttingen), Heinz Osiewacz (Institute of Molecular Biosciences, Goethe-University Frankfurt/M.), Bernd Ludwig (Institute of Biochemistry, Goethe-University Frankfurt/M.), and David M. Köller (Oregon Health & Science University, Portland, OR) for providing antibodies, reagents and yeast strains. The SFB 472 Molecular Bioenergetics and the Center for Membrane Proteomics (CMP) supported this work.

References

[1] C.F. Higgins, ABC transporters: from microorganisms to man, Annu. Rev. Cell. Biol. 8 (1992) 67–113.
[2] M. Dean, A. Rzhetsky, R. Allikmets, The human ATP-binding cassette (ABC) transporter superfamily, Genome Res. 11 (2001) 1156–1166.
[3] B. Goodner, G. Hinkle, S. Gattung, N. Miller, M. Blanchard, B. Qurollo, B.S. Goldman, Y. Cao, M. Askenazi, C. Halling, L. Mullin, K. Houmiel, J. Gordon, M. Vaudin, O. Iartchouk, A. Epp, F. Liu, C. Wollam, M. Allinger, D. Doughty, C. Scott, C. Lappas, B. Markelz, C. Flanagan, C. Crowell, J. Gurson, C. Lomo, C. Sear, G. Strub, C. Cielo, S. Slater, Genome sequence of the plant pathogen and biotechnology agent *Agrobacterium tumefaciens* C58, Science 294 (2001) 2323–2328.
[4] D.W. Wood, J.C. Setubal, R. Kaul, D.E. Monks, J.P. Kitajima, V.K. Okura, Y. Zhou, L. Chen, G.E. Wood, N.F. Almeida Jr., L. Woo, Y. Chen, I.T. Paulsen, J.A. Eisen, P.D. Karp, D. Bovee Sr., P. Chapman, J. Clendenning, G. Deatherage, W. Gillet, C. Grant, T. Kutyavin, R. Levy, M.J. Li, E. McClelland, A. Palmieri, C. Raymond, G. Rouse, C. Saenphimmachak, Z. Wu, P. Romero, D. Gordon, S. Zhang, H. Yoo, Y. Tao, P. Biddle, M. Jung, W. Krespan, M. Perry, B. Gordon-Kamm, L. Liao, S. Kim, C. Hendrick, Z.Y. Zhao, M. Dolan, F. Chumley, S.V. Tingey, J.F. Tomb, M.P. Gordon, M.V. Olson, E.W. Nester, The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58, Science 294 (2001) 2317–2323.
[5] Y. Gbelska, J.J. Krijger, K.D. Breunig, Evolution of gene families: the multidrug resistance transporter genes in five related yeast species, FEMS Yeast Res. 6 (2006) 345–355.
[6] H. Jungwirth, K. Kuchler, Yeast ABC transporters — a tale of sex, stress, drugs and aging, FEBS Lett. 580 (2006) 1131–1138.
[7] A.L. Davidson, J. Chen, ATP-binding cassette transporters in bacteria, Annu. Rev. Biochem. 73 (2004) 241–268.
[8] M. Orth, A.H. Schapira, Mitochondrial involvement in Parkinson's disease, Neurochem. Int. 40 (2002) 533–541.
[9] M. Hashimoto, E. Rockenstein, L. Crews, E. Masliah, Role of protein aggregation in mitochondrial dysfunction and neurodegeneration in Alzheimer's and Parkinson's diseases, Neuromol. Med. 4 (2003) 21–36.
[10] D.C. Wallace, The mitochondrial genome in human adaptive radiation and disease: on the road to therapeutics and performance enhancement, Gene 354 (2005) 169–180.
[11] D.C. Wallace, Mitochondrial diseases in man and mouse, Science 283 (1999) 1482–1488.
[12] S. Bekri, G. Kispal, H. Lange, E. Fitzsimons, J. Tolmie, R. Lill, D.F. Bishop, Human ABC7 transporter: gene structure and mutation causing X-linked sideroblastic anemia with ataxia with disruption of cytosolic iron–sulfur protein maturation, Blood 96 (2000) 3256–3264.
[13] J. Boultwood, A. Pellagatti, M. Nikpour, B. Pushkaran, C. Fidler, H. Cattan, T.J. Littlewood, L. Malcovati, M.G. Della Porta, M. Jadersten, S. Killick, A. Giagounidis, D. Bowen, E. Hellstrom-Lindberg, M. Cazzola, J.S. Wainscoat, The role of the iron transporter ABCB7 in refractory anemia with ring sideroblasts, PLoS ONE 3 (2008) e1970.
[14] H. Ardehali, B. O'Rourke, E. Marban, Cardioprotective role of the mitochondrial ATP-binding cassette protein 1, Circ. Res. 97 (2005) 740–742.
[15] G. Kispal, P. Csere, C. Prohl, R. Lill, The mitochondrial proteins Atm1p and Nfs1p are essential for biogenesis of cytosolic Fe/S proteins, EMBO J. 18 (1999) 3981–3989.
[16] L. Young, K. Leonhard, T. Tatsuta, J. Trowsdale, T. Langer, Role of the ABC transporter Mdl1 in peptide export from mitochondria, Science 291 (2001) 2135–2138.

[17] K.P. Locher, A.T. Lee, D.C. Rees, The *E. coli* BtuCD structure: a framework for ABC transporter architecture and mechanism, Science 296 (2002) 1091–1098.
[18] M.L. Oldham, D. Khare, F.A. Quiocho, A.L. Davidson, J. Chen, Crystal structure of a catalytic intermediate of the maltose transporter, Nature 450 (2007) 515–521.
[19] M. Chloupkova, S.K. Reaves, L.M. LeBard, D.M. Koeller, The mitochondrial ABC transporter Atm1p functions as a homodimer, FEBS Lett. 569 (2004) 65–69.
[20] M. Hofacker, S. Gompf, A. Zutz, C. Presenti, W. Haase, C. van der Does, K. Model, R. Tampé, Structural and functional fingerprint of the mitochondrial ATP-binding cassette transporter Mdl1 from *Saccharomyces cerevisiae*, J. Biol. Chem. 282 (2007) 3951–3961.
[21] N. Rayapuram, J. Hagenmuller, J.M. Grienenberger, P. Giege, G. Bonnard, AtCCMA interacts with AtCcmB to form a novel mitochondrial ABC transporter involved in cytochrome *c* maturation in *Arabidopsis*, J. Biol. Chem. 282 (2007) 21015–21023.
[22] J. Koch, R. Guntrum, S. Heintke, C. Kyritsis, R. Tampé, Functional dissection of the transmembrane domains of the transporter associated with antigen processing (TAP), J. Biol. Chem. 279 (2004) 10142–10147.
[23] S. Schrodt, J. Koch, R. Tampé, Membrane topology of the transporter associated with antigen processing (TAP1) within an assembled functional peptide-loading complex, J. Biol. Chem. 281 (2006) 6455–6462.
[24] J.C. Wolters, R. Abele, R. Tampé, Selective and ATP-dependent translocation of peptides by the homodimeric ATP binding cassette transporter TAP-like (ABCB9), J. Biol. Chem. 280 (2005) 23631–23636.
[25] R.J. Dawson, K.P. Locher, Structure of a bacterial multidrug ABC transporter, Nature 443 (2006) 180–185.
[26] A. Ward, C.L. Reyes, J. Yu, C.B. Roth, G. Chang, Flexibility in the ABC transporter MsbA: alternating access with a twist, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 19005–19010.
[27] J.E. Walker, M. Saraste, M.J. Runswick, N.J. Gay, Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold, EMBO J. 1 (1982) 945–951.
[28] K.P. Hopfner, A. Karcher, D.S. Shin, L. Craig, L.M. Arthur, J.P. Carney, J.A. Tainer, Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily, Cell 101 (2000) 789–800.
[29] N. Karpowich, O. Martsinkevich, L. Millen, Y.R. Yuan, P.L. Dai, K. MacVey, P.J. Thomas, J.F. Hunt, Crystal structures of the MJ1267 ATP binding cassette reveal an induced-fit effect at the ATPase active site of an ABC transporter, Structure 9 (2001) 571–586.
[30] R. Gaudet, D.C. Wiley, Structure of the ABC ATPase domain of human TAP1, the transporter associated with antigen processing, EMBO J. 20 (2001) 4964–4972.
[31] P.C. Smith, N. Karpowich, L. Millen, J.E. Moody, J. Rosen, P.J. Thomas, J.F. Hunt, ATP binding to the motor domain from an ABC transporter drives formation of a nucleotide sandwich dimer, Mol. Cell 10 (2002) 139–149.
[32] J. Chen, G. Lu, J. Lin, A.L. Davidson, F.A. Quiocho, A tweezers-like motion of the ATP-binding cassette dimer in an ABC transport cycle, Mol. Cell 12 (2003) 651–661.
[33] G. Verdon, S.V. Albers, B.W. Dijkstra, A.J. Driessen, A.M. Thunnissen, Crystal structures of the ATPase subunit of the glucose ABC transporter from Sulfolobus solfataricus: nucleotide-free and nucleotide-bound conformations, J. Mol. Biol. 330 (2003) 343–358.
[34] Y.R. Yuan, S. Blecker, O. Martsinkevich, L. Millen, P.J. Thomas, J.F. Hunt, The crystal structure of the MJ0796 ATP-binding cassette. Implications for the structural consequences of ATP hydrolysis in the active site of an ABC transporter, J. Biol. Chem. 276 (2001) 32313–32321.
[35] J.E. Moody, L. Millen, D. Binns, J.F. Hunt, P.J. Thomas, Cooperative, ATP-dependent association of the nucleotide binding cassettes during the catalytic cycle of ATP-binding cassette transporters, J. Biol. Chem. 277 (2002) 21111–21114.
[36] L. Schmitt, R. Tampé, Structure and mechanism of ABC transporters, Curr. Opin. Struct. Biol. 12 (2002) 754–760.
[37] E.E. Fetsch, A.L. Davidson, Vanadate-catalyzed photocleavage of the signature motif of an ATP-binding cassette (ABC) transporter, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 9685–9690.
[38] J. Zaitseva, S. Jenewein, T. Jumpertz, I.B. Holland, L. Schmitt, H662 is the linchpin of ATP hydrolysis in the nucleotide-binding domain of the ABC transporter HlyB, EMBO J. 24 (2005) 1901–1910.
[39] L.W. Hung, I.X. Wang, K. Nikaido, P.Q. Liu, G.F. Ames, S.H. Kim, Crystal structure of the ATP-binding subunit of an ABC transporter, Nature 396 (1998) 703–707.
[40] L. Schmitt, H. Benabdelhak, M.A. Blight, I.B. Holland, M.T. Stubbs, Crystal structure of the nucleotide-binding domain of the ABC-transporter haemolysin B: identification of a variable region within ABC helical domains, J. Mol. Biol. 330 (2003) 333–342.
[41] H.A. Lewis, S.G. Buchanan, S.K. Burley, K. Conners, M. Dickey, M. Dorwart, R. Fowler, X. Gao, W.B. Guggino, W.A. Hendrickson, J.F. Hunt, M.C. Kearins, D. Lorimer, P.C. Maloney, K.W. Post, K.R. Rajashankar, M.E. Rutter, J.M. Sauder, S. Shriver, P.H. Thibodeau, P.J. Thomas, M. Zhang, X. Zhao, S. Emtage, Structure of nucleotide-binding domain 1 of the cystic fibrosis transmembrane conductance regulator, EMBO J. 23 (2004) 282–293.
[42] E. Janas, M. Hofacker, M. Chen, S. Gompf, C. van der Does, R. Tampé, The ATP hydrolysis cycle of the nucleotide-binding domain of the mitochondrial ATP-binding cassette transporter Mdl1p, J. Biol. Chem. 278 (2003) 26862–26869.
[43] C. van der Does, C. Presenti, K. Schulze, S. Dinkelaker, R. Tampé, Kinetics of the ATP hydrolysis cycle of the nucleotide-binding domain of Mdl1 studied by a novel site-specific labeling technique, J. Biol. Chem. 281 (2006) 5694–5701.
[44] C. van der Does, R. Tampé, How do ABC transporters drive transport? Biol. Chem. 385 (2004) 927–933.
[45] K. Hollenstein, R.J. Dawson, K.P. Locher, Structure and mechanism of ABC transporter proteins, Curr. Opin. Struct. Biol. 17 (2007) 412–418.
[46] M.L. Oldham, A.L. Davidson, J. Chen, Structural insights into ABC transporter mechanism, Curr. Opin. Struct. Biol. 18 (2008) 726–733.
[47] A. Sickmann, J. Reinders, Y. Wagner, C. Joppich, R. Zahedi, H.E. Meyer, B. Schonfisch, I. Perschil, A. Chacinska, B. Guiard, P. Rehling, N. Pfanner, C. Meisinger, The proteome of *Saccharomyces cerevisiae* mitochondria, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 13207–13212.
[48] S.W. Taylor, E. Fahy, B. Zhang, G.M. Glenn, D.E. Warnock, S. Wiley, A.N. Murphy, S.P. Gaucher, R.A. Capaldi, B.W. Gibson, S.S. Ghosh, Characterization of the human heart mitochondrial proteome, Nat. Biotechnol. 21 (2003) 281–286.
[49] J.M. Herrmann, W. Neupert, Protein transport into mitochondria, Curr. Opin. Microbiol. 3 (2000) 210–214.
[50] R.E. Jensen, C.D. Dunn, Protein import into and across the mitochondrial inner membrane: role of the TIM23 and TIM22 translocons, Biochim. Biophys. Acta 1592 (2002) 25–34.
[51] N. Wiedemann, A.E. Frazier, N. Pfanner, The protein import machinery of mitochondria, J. Biol. Chem. 279 (2004) 14473–14476.
[52] N. Bolender, A. Sickmann, R. Wagner, C. Meisinger, N. Pfanner, Multiple pathways for sorting mitochondrial precursor proteins, EMBO.Rep. 9 (2008) 42–49.
[53] P. Csere, R. Lill, G. Kispal, Identification of a human mitochondrial ABC transporter, the functional orthologue of yeast Atm1p, FEBS Lett. 441 (1998) 266–270.
[54] S.A. Graf, S.E. Haigh, E.D. Corson, O.S. Shirihai, Targeting, import, and dimerization of a mammalian mitochondrial ATP binding cassette (ABC) transporter, ABCB10 (ABC-me), J. Biol. Chem. 279 (2004) 42954–42963.
[55] S. Gompf, A. Zutz, M. Hofacker, W. Haase, C. van der Does, R. Tampé, Switching of the homooligomeric ATP-binding cassette transport complex MDL1 from post-translational mitochondrial import to endoplasmic reticulum insertion, FEBS J. 274 (2007) 5298–5310.
[56] E. Miyazaki, Y. Kida, K. Mihara, M. Sakaguchi, Switching the sorting mode of membrane proteins from cotranslational endoplasmic reticulum targeting to posttranslational mitochondrial import, Mol. Biol. Cell. 16 (2005) 1788–1799.
[57] S. Kanaji, J. Iwahashi, Y. Kida, M. Sakaguchi, K. Mihara, Characterization of the signal that directs Tom20 to the mitochondrial outer membrane, J. Cell Biol. 151 (2000) 277–288.
[58] P.C. Krishnamurthy, G. Du, Y. Fukuda, D. Sun, J. Sampath, K.E. Mercer, J. Wang, B. Sosa-Pineda, K.G. Murti, J.D. Schuetz, Identification of a mammalian mitochondrial porphyrin transporter, Nature 443 (2006) 586–589.
[59] J.K. Paterson, S. Shukla, C.M. Black, T. Tachiwada, S. Garfield, S. Wincovitch, D.N. Ernst, A. Agadir, X. Li, S.V. Ambudkar, G. Szakacs, S. Akiyama, M.M. Gottesman, Human ABCB6 localizes to both the outer mitochondrial membrane and the plasma membrane, Biochemistry 46 (2007) 9443–9452.
[60] M. Tsuchida, Y. Emi, Y. Kida, M. Sakaguchi, Human ABC transporter isoform B6 (ABCB6) localizes primarily in the *Golgi* apparatus, Biochem. Biophys. Res. Commun. 369 (2008) 369–375.
[61] D. Rapaport, Finding the right organelle. Targeting signals in mitochondrial outer-membrane proteins, EMBO Rep. 4 (2003) 948–952.
[62] J. Leighton, G. Schatz, An ABC transporter in the mitochondrial inner membrane is required for normal growth of yeast, EMBO J. 14 (1995) 188–195.
[63] G. Kispal, P. Csere, B. Guiard, R. Lill, The ABC transporter Atm1p is required for mitochondrial iron homeostasis, FEBS Lett. 418 (1997) 346–350.
[64] M. Chloupkova, L.S. LeBard, D.M. Koeller, MDL1 is a high copy suppressor of ATM1: evidence for a role in resistance to oxidative stress, J. Mol. Biol. 331 (2003) 155–165.
[65] J. Dong, R. Lai, K. Nielsen, C.A. Fekete, H. Qiu, A.G. Hinnebusch, The essential ATP-binding cassette protein RLI1 functions in translation by promoting preinitiation complex assembly, J. Biol. Chem. 279 (2004) 42157–42168.
[66] A. Hausmann, B. Samans, R. Lill, U. Muhlenhoff, Cellular and mitochondrial remodeling upon defects in iron–sulfur protein biogenesis, J. Biol. Chem. 283 (2008) 8318–8330.
[67] G. Kuhnke, K. Neumann, U. Muhlenhoff, R. Lill, Stimulation of the ATPase activity of the yeast mitochondrial ABC transporter Atm1p by thiol compounds, Mol. Membr. Biol. 23 (2006) 173–184.
[68] N. Mitsuhashi, T. Miki, H. Senbongi, N. Yokoi, H. Yano, M. Miyazaki, N. Nakajima, T. Iwanaga, Y. Yokoyama, T. Shibata, S. Seino, MTABC3, a novel mitochondrial ATP-binding cassette protein involved in iron homeostasis, J. Biol. Chem. 275 (2000) 17536–17540.
[69] S. Savary, R. Allikmets, F. Denizot, M.F. Luciani, M.G. Mattei, M. Dean, G. Chimini, Isolation and chromosomal mapping of a novel ATP-binding cassette transporter conserved in mouse and human, Genomics 41 (1997) 275–278.
[70] C. Pondarre, B.B. Antiochos, D.R. Campagna, S.L. Clarke, E.L. Greer, K.M. Deck, A. McDonald, A.P. Han, A. Medlock, J.L. Kutok, S.A. Anderson, R.S. Eisenstein, M.D. Fleming, The mitochondrial ATP-binding cassette transporter Abcb7 is essential in mice and participates in cytosolic iron–sulfur cluster biogenesis, Hum. Mol. Genet. 15 (2006) 953–964.
[71] C. Pondarre, D.R. Campagna, B. Antiochos, L. Sikorski, H. Mulhern, M.D. Fleming, Abcb7, the gene responsible for X-linked sideroblastic anemia with ataxia, is essential for hematopoiesis, Blood 109 (2007) 3567–3569.
[72] S. Taketani, K. Kakimoto, H. Ueta, R. Masaki, T. Furukawa, Involvement of ABC7 in the biosynthesis of heme in erythroid cells: interaction of ABC7 with ferrochelatase, Blood 101 (2003) 3274–3280.
[73] P. Cavadini, G. Biasiotto, M. Poli, S. Levi, R. Verardi, I. Zanella, M. Derosas, R. Ingrassia, M. Corrado, P. Arosio, RNA silencing of the mitochondrial ABCB7 transporter in HeLa cells causes an iron-deficient phenotype with mitochondrial iron overload, Blood 109 (2007) 3552–3559.
[74] G. Szakacs, J.P. Annereau, S. Lababidi, U. Shankavaram, A. Arciello, K.J. Bussey, W.

Reinhold, Y. Guo, G.D. Kruh, M. Reimers, J.N. Weinstein, M.M. Gottesman, Predicting drug sensitivity and resistance: profiling ABC transporter genes in cancer cells, Cancer Cell 6 (2004) 129–137.
[75] D.G. Kakhniashvili, L.A. Bulla Jr., S.R. Goodman, The human erythrocyte proteome: analysis by ion trap mass spectrometry, Mol. Cell Proteomics 3 (2004) 501–509.
[76] R.C. Beier, Natural pesticides and bioactive components in foods, Rev. Environ. Contam. Toxicol. 113 (1990) 47–137.
[77] M. Dean, R. Allikmets, B. Gerrard, C. Stewart, A. Kistler, B. Shafer, S. Michaelis, J. Strathern, Mapping and sequencing of two yeast genes belonging to the ATP-binding cassette superfamily, Yeast 10 (1994) 377–383.
[78] S. Uebel, W. Kraas, S. Kienle, K.H. Wiesmüller, G. Jung, R. Tampé, Recognition principle of the TAP transporter disclosed by combinatorial peptide libraries, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 8976–8981.
[79] F.J. Detmers, F.C. Lanfermeijer, R. Abele, R.W. Jack, R. Tampé, W.N. Konings, B. Poolman, Combinatorial peptide libraries reveal the ligand-binding mechanism of the oligopeptide receptor OppA of *Lactococcus lactis*, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 12487–12492.
[80] D. Galluhn, T. Langer, Reversible assembly of the ATP-binding cassette transporter Mdl1 with the F_1F_0-ATP synthase in mitochondria, J. Biol. Chem. 279 (2004) 38338–38345.
[81] F. Zhang, D.L. Hogue, L. Liu, C.L. Fisher, D. Hui, S. Childs, V. Ling, M-ABC2, a new human mitochondrial ATP-binding cassette membrane protein, FEBS.Lett. 478 (2000) 89–94.
[82] M.A. Burke, H. Ardehali, Mitochondrial ATP-binding cassette proteins, Transl. Res. 150 (2007) 73–80.
[83] O.S. Shirihai, T. Gregory, C. Yu, S.H. Orkin, M.J. Weiss, ABC-me: a novel mitochondrial transporter induced by GATA-1 during erythroid differentiation, EMBO J. 19 (2000) 2492–2502.
[84] D. Lockshon, L.E. Surface, E.O. Kerr, M. Kaeberlein, B.K. Kennedy, The sensitivity of yeast mutants to oleic acid implicates the peroxisome and other processes in membrane function, Genetics 175 (2007) 77–91.
[85] D.L. Hogue, L. Liu, V. Ling, Identification and characterization of a mammalian mitochondrial ATP-binding cassette membrane protein, J. Mol. Biol. 285 (1999) 379–389.
[86] H. Ardehali, Z. Chen, Y. Ko, R. Mejia-Alvarez, E. Marban, Multiprotein complex containing succinate dehydrogenase confers mitochondrial ATP-sensitive K^+ channel activity, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 11880–11885.
[87] J.D. Thompson, D.G. Higgins, T.J. Gibson, CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, Nucleic Acids Res. 22 (1994) 4673–4680.
[88] A. Sali, T.L. Blundell, Comparative protein modelling by satisfaction of spatial restraints, J. Mol. Biol. 234 (1993) 779–815.
[89] N. Guex, M.C. Peitsch, SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling, Electrophoresis 18 (1997) 2714–2723.
[90] H. Schägger, Blue-native gels to isolate protein complexes from mitochondria, Methods Cell Biol. 65 (2001) 231–244.
[91] I. Wittig, J. Velours, R. Stuart, H. Schägger, Characterization of domain interfaces in monomeric and dimeric ATP synthase, Mol. Cell Proteomics 7 (2008) 995–1004.